Trehalose protects against spinal cord ischemia in rabbits

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Objective: **This study tested to see if trehalose, a cytoprotective disaccharide, protects against spinal cord ischemia in a rabbit model.**

Methods: **The infrarenal aorta was mobilized in four groups of 10 rabbits. In groups I, II, and III, it was clamped proximally and distally for 20 minutes. In group I, the clamped aorta was infused at 2.5 L/min for 2 hours with lactated Ringer's (LR) solution. In group II, the clamped aorta was infused with 5% trehalose in LR. LR was administered intravenously (2.0 mL/min) in groups I and II starting 30 minutes before clamping. In group III, 5% trehalose in LR was infused intravenously only. Group IV was a sham-operated control group without aortic clamping. At 8, 24, and 48 hours after reperfusion, hind limb function was scored using the Tarlov score (paralysis = 0, perceptible joint movement = 1, good joint movement but unable to stand = 2, able to walk = 3, normal = 4). Histologic analysis and electron microscopy were performed on anterior horn cells.**

Results: **The Tarlov scores in groups I, II, and III were, respectively, 1.1 ± 1.4, 3.5 ± 0.5, and 2.9 ± 0.9 at 8 hours; 0.8 ± 1.2, 3.9 ± 0.3, and 2.9 ± 0.9 at 24 hours; and 0.6 ± 0.7, 3.9 ± 0.3, and 2.7 ± 0.9 at 48 hours after reperfusion. Group IV scores were normal (4 ± 0) at all assessments. These scores were higher in groups II and III than in group I ($P < .01$) at all assessments. Scores at 24 and 48 hours were higher in group II than in group III ($P < .05$). In group III, delayed paraparesis developed in one rabbit at 24 hours and in two more at 48 hours. Histopathologic analysis showed the number of normal neurons was higher in groups II ($P < .0001$), III ($P = .006$), and IV ($P < .0001$) vs group I. Electron microscopy confirmed preserved neuronal cell ultrastructure in rabbits with normal limb function.**

Conclusions: **Transaortic trehalose infusion was protective against paraplegia, whereas intravenous trehalose reduced spinal cord ischemia. This study was preliminary and further studies are needed. (J Vasc Surg 2014;60:490-6.)**

Clinical Relevance: **Spinal cord injury after surgical repair of the thoracic or thoracoabdominal aorta is a disastrous complication. Recently, many kinds of adjunctive therapy have been reported to be successful, including our previous reports of cold saline and free-radical scavengers in a rabbit model of spinal cord ischemia; however, some rabbits still became paraplegic. The total prevention of paraplegia due to spinal cord ischemia may require the development of a more effective adjunct. Trehalose, a unique nonreducing disaccharide, stabilizes cell membranes under various stressful conditions such as heat, freezing, osmotic shock, and dehydration. Trehalose has been used clinically as a solution for lung preservation in transplantation. This study demonstrated the protective effect of a trehalose infusion into the clamped segment of the aorta or transvenously to prevent spinal cord damage after spinal cord ischemia in an animal model. Further research is needed, but the results of this study may be applicable not only to open surgery but also to thoracoabdominal endovascular repair.**

Paraplegia caused by ischemic spinal cord injury is one of the most serious complications after open surgery and endovascular repair of thoracoabdominal aortic aneurysms. The incidence of paraplegia ranges from 2% to 16% in open surgery[1-4] and may be related to spinal cord malperfusion during periods of hypotension or aortic cross-clamping. Endovascular surgery is superior to open surgery in that it is associated with a lower incidence of paraplegia, but it does not eliminate the complication.[5]

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Author conflict of interest: none.

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The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.

0741-5214/$36.00

http://dx.doi.org/10.1016/j.jvs.2013.06.078

Adjunctive procedures for spinal cord protection have been introduced, including cerebrospinal fluid drainage, distal perfusion of the aorta, or use of steroids and barbiturates, and these have reduced the incidence of paraplegia.[3,6,7] Recently, many kinds of adjunctive therapy have been reported[8] to be successful, including our previous reports of cold saline and free-radical scavengers in a rabbit model of spinal cord ischemia.[9,10] Despite the use of these adjuncts, some rabbits still became paraplegic. The total prevention of paraplegia due to spinal cord ischemia may require the development of a more effective adjunct.

Trehalose is a unique disaccharide composed of two glucose molecules connected through an α, α-1,1 linkage. Recent reports have shown that trehalose protects *Drosophila* and mammalian cells from anoxic stress.[11] In the clinical setting, a solution containing trehalose has been used for lung preservation in transplantation. The transplant was flushed with the solution, and respiratory function was preserved after an ischemic time of >4 hours.[12]

In the present study, we designed a simple procedure to protect the spinal cord against ischemia using trehalose, a highly soluble sugar molecule. We then investigated the degree of protection resulting from the local or systemic administration of trehalose in a rabbit model of spinal cord injury.

METHODS

Animal preparation. Animal care and all procedures were performed in compliance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press, Washington DC, revised 1996). This study protocol was approved by the Research Facilities for Laboratory Animal Science of the Hiroshima University School of Medicine.

Anesthesia and monitoring. Japanese White rabbits (2.5-3.0 kg) were used in this experiment. General anesthesia was induced with an intramuscular injection of ketamine at a dose of 50 mg/kg. An ear vein catheter was inserted for the administration of additional drugs. Cefazolin (10 mg/kg) was injected through the catheter before any surgical procedures were performed.

After a tracheostomy, the rabbits were ventilated mechanically with a fraction of inspired oxygen of 1.0 and isoflurane (1.5% to 2.0%) to maintain a surgical plane of anesthesia. Adequate ventilation was monitored by periodic blood gas analysis.

A JMS C3 arterial catheter cutdown tube (JMS Company, Hiroshima, Japan) was inserted into the right axillary artery and the right femoral artery for blood pressure monitoring and for obtaining blood samples for blood gas analysis and blood glucose levels. A three-lead electrocardiogram was also monitored continuously. The rectal temperature was continuously monitored and maintained at 38.0° ± 0.5°C using a warming blanket.

Surgical procedures. With the rabbit in the right lateral decubitus position, a flank skin incision parallel to the spine was made on the left side. The abdominal aorta was exposed below the left renal artery and above the iliac bifurcation retroperitoneally and encircled with sutures. A 24-gauge catheter was inserted into the aorta 2 cm above the iliac bifurcation and connected to an extension tube. After the intravenous administration of heparin (100 U/kg), the abdominal aorta just distal to the left renal artery and just proximal to the iliac bifurcation was cross-clamped using vascular clamps. This segment, which included the third through sixth lumber artery, was isolated for 20 minutes to produce spinal cord ischemia.

The 20-minute cross-clamp time was determined from a series of preliminary experiments in which 15 rabbits were divided into three groups of five rabbits each with clamping times of 10, 15, and 20 minutes. The abdominal aorta just distal to the left renal artery and the aortic bifurcation were both clamped. At 2 days after reperfusion, all of the rabbits in the group clamped for 20 minutes were paraplegic.

Drug delivery. Forty rabbits were randomly divided into four groups (I, II, III, and IV), with 10 animals in each group. In group I, lactated Ringer's (LR) solution was infused into the clamped segment of aorta at 2.5 mL/min. In group II, 5% trehalose with LR was infused into the clamped segment of aorta, as in group I. In groups I and II, LR was infused intravenously at 2.0 mL/min for 2 hours starting 30 minutes before clamping. In group III, 5% trehalose with LR was infused intravenously at 2.0 mL/min for 2 hours starting 30 minutes before clamping, as in group I. Group IV was a sham-operated control group without aortic clamping. The concentration of trehalose was selected based on a previous study that reported the optimal concentration of trehalose for lung preservation.[13] The temperature of all these solutions was maintained at 37°C by a thermostatic bath.

The catheter inserted into the clamped aortic segment was immediately removed after the injection of the solution, and the point of catheter entry was sutured with 8-0 Prolene (Ethicon Inc, Somerville, NJ). After the aorta was declamped, the abdomen was closed, and all catheters were withdrawn. The animals were extubated after they recovered from anesthesia.

Neurologic study. Each rabbit's neurologic status was assessed at 8, 24, and 48 hours after reperfusion by an observer blinded to the protocol used for each animal. The motor function of the hind limbs was graded using the Tarlov score: 0, no movement of the hind limbs; 1, perceptible movement of the joints of the hind limbs; 2, good movement of the joints but an inability to stand; 3, ability to stand and walk; and 4, complete recovery.[14]

Histopathologic study. Eight rabbits in each group were euthanized after the completion of the neurologic evaluation at 48 hours. Sectioned specimens from the lumber spinal cord at the level of L4 to L6 that was perfused by the clamped aorta were stained with hematoxylin and eosin and evaluated for ischemic pathology using standard light microscopy. Neuronal ischemic injury was evaluated at original magnification ×200 by an observer blinded to the treatment groups.

The number of normal motor neurons in the anterior horn of the spinal cord (anterior to a line drawn through the central canal perpendicular to the anterior median fissure) was counted in five slices of specimens from each animal and was averaged. Ischemic neurons were identified by a shrunken and triangular-shaped cell body, an eosinophilic cytoplasm with the loss of Nissl granules, a nucleus that was triangular and pyknotic, and finally, a smaller neuron that underwent homogenization.

Electron microscopy. Two rabbits in each group were anesthetized 48 hours after the experiment and transcardially perfused with saline solution, quickly followed by 2% glutaraldehyde in 0.1M phosphate buffer (pH 7.6). After perfusion, the L4 to L6 segment of the spinal cord was removed, and slabs were placed in 2% glutaraldehyde with phosphate-buffered saline at 4°C. Slabs were washed in phosphate buffer and incubated with 1% osmium tetroxide (in phosphate buffer for 1 hour). Slabs were dehydrated in a graded ethanol series. Infiltration was accomplished with the use of a series of propylene oxide

Table. Proximal and distal pressure and blood glucose level during the operation

Variable[a]	*Group I*	*Group II*	*Group III*	*Group IV*
Arterial pressure				
Proximal				
Before clamp, mm Hg	108 ± 13.5	112.9 ± 8.0	110 ± 6.2	114.0 ± 10.1
During clamp, mm Hg	100 ± 10.0	109.6 ± 9.3	112.3 ± 5.7	113.4 ± 4.3
After declamp, mm Hg	89.3 ± 7.1	94.4 ± 8.4	106.0 ± 5.3	90.4 ± 12.1
Distal				
Before clamp, mm Hg	98.5 ± 4.3	103.8 ± 10.8	97.0 ± 8.7	94.4 ± 4.0
During clamp, mm Hg	17.6 ± 5.8	17.9 ± 7.9	27.2 ± 11.3	107.1 ± 12.5[b]
After clamp, mm Hg	73.9 ± 8.7	67.7 ± 14.0	67.3 ± 7.8	90.4 ± 7.3
Blood glucose level				
Before clamp, mg/dL	110.8 ± 13.2	110.0 ± 8.4	109.0 ± 13.6	111.0 ± 17.5
After clamp, mg/dL	123.0 ± 8.8	125.1 ± 8.3	134.8 ± 25.2	104.3 ± 12.4[b]

[a]Data are shown as mean ± standard deviation.
[b]$P < .05$.

and EmBed 812 (EM Sciences, Hatfield, Pa) mixture, with slabs left in 100% EmBed 812 for 6 hours. Finally, slabs were flat embedded between ACLAR sheets (Ted Pella Inc, Redding, Calif) and dried at 60°C overnight.

Slabs were glued to blank blocks, and ultrathin sections (60-90 nm) were cut on a diamond knife, collected on copper grids, and stained with 3% uranyl acetate in 50% ethanol for 20 minutes, followed by lead citrate for 3 minutes. Sections were visualized and photographed on a Hitachi H300 transmission electron microscope (Hitachi Ltd Instruments Division, Tokyo Japan).

Statistical analysis. Results are expressed as means ± standard deviation. Two groups were compared with a Mann-Whitney *U* test. When there were more than two groups, a one-way analysis of variance was performed, followed by post-hoc testing with the Fisher protected least significant difference test to identify which group differences were significant. Statistical significance was assumed at $P < .05$.

RESULTS

Physiologic status. No signs of toxicity were observed after trehalose administration into the clamped aortic segment or intravenously. The heart rate and arterial blood gases were similar among the four groups. Data on arterial pressure and blood glucose levels are reported in the Table. No significant difference was noted in the proximal and distal arterial pressure among the groups during the operation. In groups I, II, and III, blood glucose levels after aortic declamping were higher than those before clamping ($P < .05$). The increase in blood glucose levels after declamping was not significantly different among groups I, II, and III.

Neurologic outcome. The neurologic results are summarized in Fig 1. The Tarlov scores in groups I, II, III, and IV were, respectively: 1.1 ± 1.4, 3.5 ± 0.5, 2.9 ± 0.9 and 4.0 ± 0 at 8 hours; 0.8 ± 1.2, 3.9 ± 0.3, 2.9 ± 0.9 and 4.0 ± 0 at 24 hours; and 0.6 ± 0.7, 3.9 ± 0.3, 2.7 ± 0.9 and 4.0 ± 0 at 48 hours after reperfusion. The scores at all time points postoperatively were significantly higher in groups II and III than in group I ($P < .01$). The scores at 24 and 48 hours after reperfusion were significantly higher in group II than in group III ($P < .05$). Delayed paraparesis was observed in group I in one rabbit at 48 hours and in group III in one rabbit at 24 hours and in two rabbits at 48 hours.

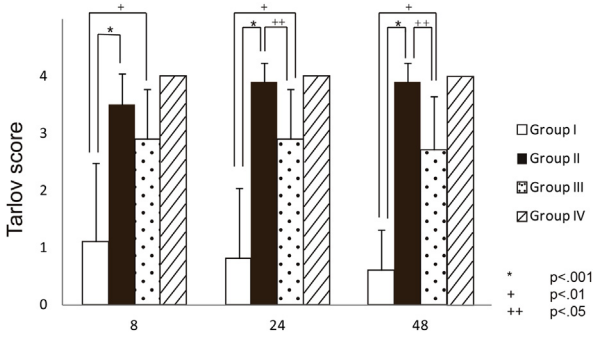

Fig 1. Changes in the Tarlov score at each time point. The score was significantly higher in groups II and III than in group I at 8, 24, and 48 hours and was significantly higher in group II than in group III at 24 and 48 hours. The *error bars* indicate the standard deviation.

Histopathologic assessment. Fig 2 shows the typical histopathologic findings of the spinal cord from a group I rabbit with paraplegia, a group II rabbit with normal neurologic function, a group III rabbit with Tarlov 2 paraparesis, and a group IV rabbit with normal neurologic function. Necrosis in the anterior horn was detected in group I (Fig 2, *A*), and the motor neurons were disrupted, with evidence of vacuolation in the cytoplasm and loss of Nissl granules. Inflammatory cell accumulation was detected. In group II (Fig 2, *B*), the motor neurons had a normal appearance and minimal inflammatory cell accumulation was detected. Normal and necrotic motor neurons were both present in group III (Fig 2, *C*). In group IV (Fig 2, *D*), all of the motor neuron cells had a normal appearance, without inflammatory changes.

The number of normal motor neurons in the anterior horn of the spinal cord was 5.8 ± 2.2, 48.3 ± 4.9,

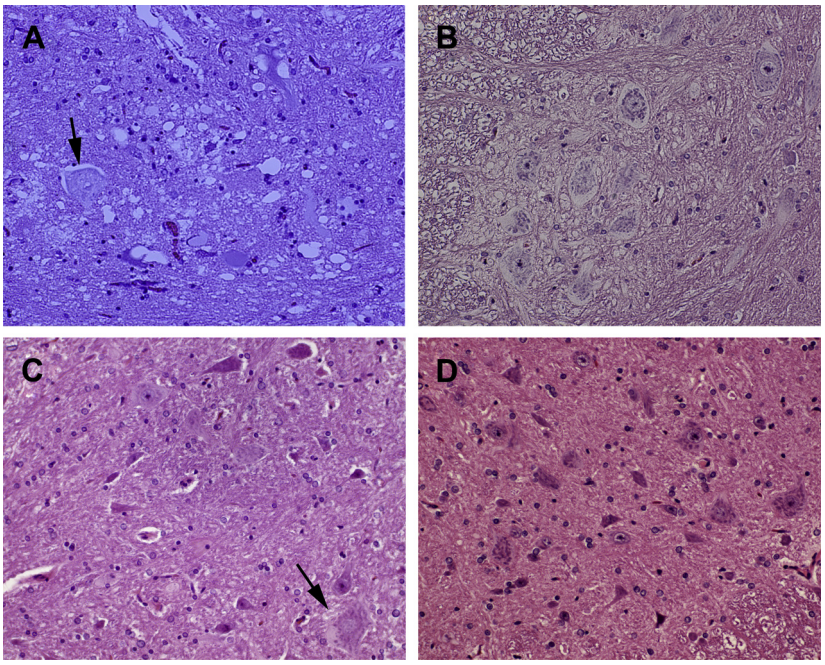

Fig 2. Typical histopathologic findings in the spinal cord 48 hours after reperfusion (hematoxylin and eosin stain; original magnification ×200). **A,** In group I, ~90% of motor neurons were lost. **B,** In group II, no ischemic damage was detected, and there were many motor neurons. **C,** In group III, there were intact neurons, but ~70% of motor neurons were lost. **D,** The spinal cord from a sham-operated control rabbit was intact. The *arrows* indicate normal motor neurons, and the arrowheads, necrotic neurons.

15.9 ± 2.8, and 63.2 ± 8.1 at 48 hours after reperfusion in groups I, II, III, and IV, respectively. The number of normal neurons was significantly higher in groups II and IV than in group I ($P < .0001$). Moreover, the number of normal neurons in group III was significantly higher than that in group I ($P = .006$; Fig 3).

Electron microscopy. In groups I, II, and III, rabbits with paraplegia or paraparesis, or both, and rabbits with normal limb function were selected and their spinal cord specimens were examined by electron microscopy. In group IV, two rabbits with normal limb function were examined. Fig 4 shows the typical transmission electron microscopic findings of neuronal cells in the anterior horn of the spinal cord from a rabbit in group I with paraplegia, a group II rabbit with normal neurologic function, a group III rabbit with Tarlov 2 paraparesis, and a group IV rabbit with normal neurologic function.

In group I, parts of the neuronal cell membranes were fragmented, and cytoplasmic elements were sparse. The mitochondria of neuronal cells showed swelling and disruption as well. All of these findings indicated necrosis (Fig. 4, *A* and *B*). Cell membranes in group II showed normal structure and the cytoplasmic components were intact (Fig 4, *C* and *D*). In group III, there were findings that were similar to those in groups I and II (Fig 4, *E*). Cell membranes in group IV showed normal structure, and the cytoplasmic components were intact (Fig. 4, *F*). There was no indication of apoptosis in any of the groups.

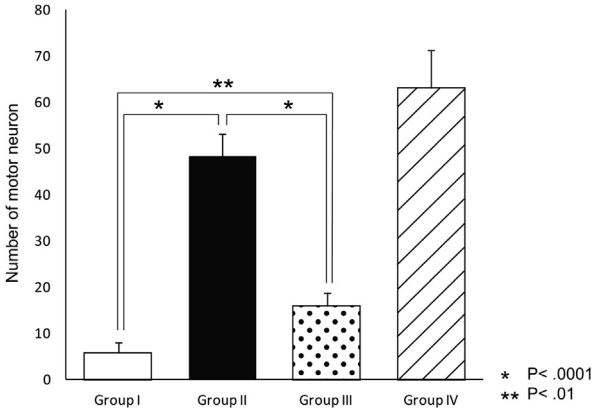

Fig 3. The number of normal neurons was significantly larger in groups II and III than in group I. Moreover, the number of normal neurons was significantly higher in group II than in group III. The number of normal neurons was significantly larger in group IV than groups I, II, and III ($P < .0001$). The *error bars* indicate the standard deviation.

DISCUSSION

This study demonstrates that a normothermic trehalose solution infused into an isolated aortic segment as well as intravenously can reduce the neurologic damage caused by spinal cord ischemia in a rabbit model.

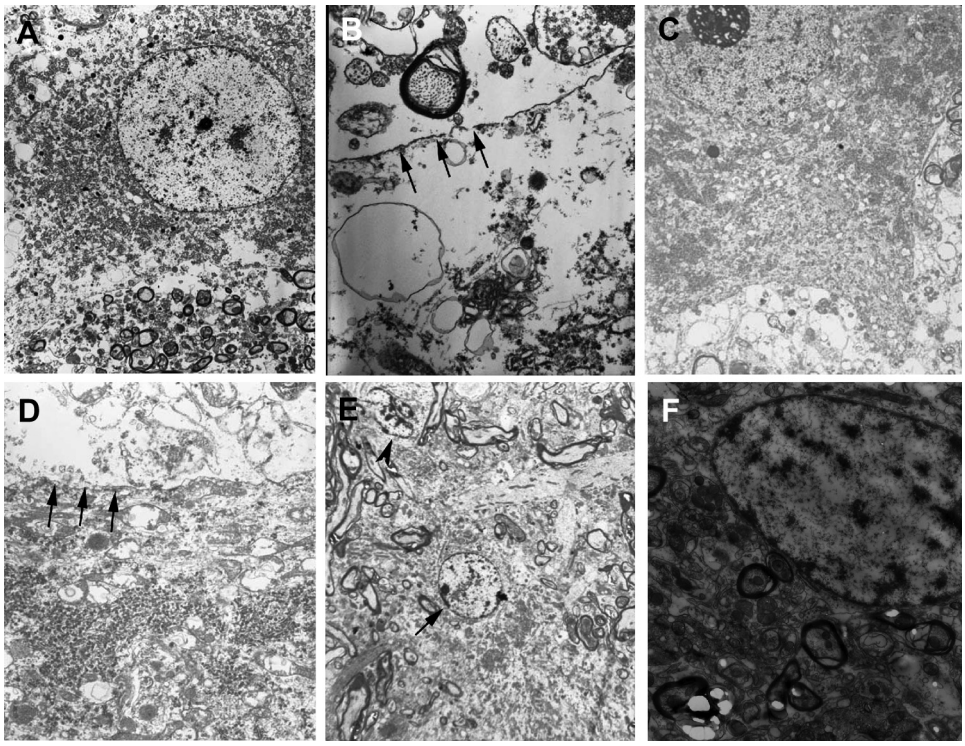

Fig 4. Typical transmission electron microscopic findings in neuronal cells from the anterior horn of the spinal cord. In group I, **(A)** necrosis of nucleus and **(B)** disruption of the cell membrane (*arrow*). In group II, **(C)** normal nucleus and cytoplasm and **(D)** normal cell membrane protecting cytoplasmic components. **E,** In group III, normal nucleus (*arrow*) and ischemic nucleus with chromatin condensation (*arrowhead*). **F,** In group IV, normal neuron cell. Images are at original magnification ×3000 **(A, C, E,** and **F)**, ×10,000 **(D)**, and ×15,000 **(B)**.

Trehalose (1-α-D-glucopyranosyl-α-D-glucopyranoside) is a nonreducing disaccharide and can be converted into glucose by hydrolysis. Trehalose is detected in abundance in many prokaryotes, fungi, yeasts, some desert plants, and the body fluid of insects.[15] Several studies have reported that trehalose has a preserving and stabilizing effect on cell membranes under severe conditions such as high temperature, freezing, osmotic shock, and dehydration.[16,17] In other studies, trehalose has been proven to be an effective compound to prevent protein aggregation in Alzheimer's disease, Huntington's disease, and prion disease in vitro.[18]

The effect of trehalose in preventing ischemia or an anoxic state has been described, but there is a paucity of data. Chen et al[11,19] reported that human HEK-293 cells transfected with the gene that synthesizes trehalose accumulated intracellular trehalose during hypoxia. They also reported that trehalose protects *Drosophila* and mammalian cells from anoxic stress. After exposure to low oxygen, the amount of ubiquitinated protein of human HEK-293 cells transfected with the trehalose gene is less than that of human HEK-293 cells without trehalose gene transfection.

In an experimental lung transplantation model, a lung preservation solution containing trehalose (ET-Kyoto solution) allowed longer lung preservation compared with other solutions that lacked trehalose.[20] In the clinical setting, the ischemic time of a donor lung preserved by ET-Kyoto solution was about 10 hours, and the partial pressure of arterial oxygen (fraction of inspired oxygen = 1.0) was maintained >400 mm Hg postoperatively, without the patient developing any significant complications.[21]

Saccharides are considered to play a role as osmotic impermeants and serve as an energy source for the organs during ischemia.[22] Disaccharides (trehalose, maltose, sucrose) and trisaccharides (raffinose, melezitose) are superior to monosaccharides (glucose, fructose), which penetrate the cell membrane relatively easily and are commonly used as osmotic agents to suppress the edema during organ preservation. Among the disaccharides, trehalose was superior to maltose for lung preservation, which may reflect that the reducing sugar maltose destabilizes the membrane by reacting with membrane proteins.[23] Given this evidence, we hypothesized that trehalose may be effective to protect the spinal cord from ischemic injury during thoracic and thoracoabdominal aortic surgery.

Before this study was started, the concentration of trehalose solution and the administration route were selected based on previous studies that used ET-Kyoto solution for lung preservation. Hirata et al[13] reported that 3.5% and 7.0% trehalose solutions both maintained acute canine lung function and protected endothelial cells

microscopically and that the 3.5% solution was superior for preservation of lung oxygenation capacity when lungs were stored for 20 hours at 4°C before transplantation. Bando et al[24] reported that an extracellular solution with 4.1% trehalose achieved high lung oxygenation capacity and low pulmonary artery pressure and maintained normal cell structure in an extracorporeal rat lung perfusion model.

In contrast to these two ex vivo studies in which trehalose solution was directly perfused into the pulmonary artery, our study was in vivo, and the trehalose solution was directly injected into the clamped segment of aorta to deliver the sugar solution to motor neurons. However, transaortic infusion is impossible in the case of endovascular repair, and intravenous infusion will be necessary in the clinical setting. In the present study, hind limb function was preserved in all of the rabbits with transaortic infusion and was preserved after intravenous infusion in four rabbits (40%), but paraparesis occurred in six (60%). Microscopic results showed that the transaortic injection group had less motor neuron injury than the sham-operated control group (49.0 vs 63.9), whereas the intravenous infusion group showed significant motor neuron loss compared with the sham-operated control group (16.0 vs 63.9). The number of intact motor neurons was significantly larger in the transaortic group than in the intravenous group. The number of intact motor neurons in the intravenous group was significantly higher than in group I (16.0 vs 5.8), where only LR was injected into the clamped segment of aorta. These results indicate that a transaortic trehalose infusion is superior to intravenous infusion but that an intravenous infusion has some potential to prevent spinal cord injury.

Previous studies have shown that neuronal injury caused by spinal cord ischemia occurs in two phases, an immediate phase and a delayed phase.[25,26] Disruption of infrarenal abdominal aortic blood flow in those studies for 15 minutes caused immediate neuronal injury that was evident after 24 to 48 hours and delayed paraplegia that became evident at 24 hours to 7 days. Immediate injury is often caused by a severe ischemic insult that results in rapid necrosis of neuronal cells. Delayed injury is caused by apoptosis or necrosis. In the present study, two rabbits in group III could stand and walk after the operation, but there was delayed paraparesis at 48 hours.

Several studies have investigated the mechanism of cytoprotection by trehalose.[27] Trehalose prevents fusion of the lipid bilayer and leakage of cellular contents by binding to the phospholipids in the lipid bilayer of the cell membrane, thereby stabilizing the structure. Moreover, trehalose concentrates surrounding water molecules close to the cell membrane and preserves its native properties. Although proving this mechanism is difficult, Fukuse et al[20] used an ex vivo rat lung model that was reperfused after 14 hours and found mitochondrial changes and swelling on electron microscopy that did not occur when lungs were perfused with a solution containing trehalose before ischemia. These results support the concept that trehalose has a protective effect on vascular endothelial cells.

Electron microscopic examination in the present study revealed swelling and disruption of the mitochondria and alterations of cell membranes of motor neurons in the control group. However, several motor neurons in group II showed preserved cellular membranes and intracellular structures with minimal changes. These differences may result from the intensity of the lipid bilayer, with or without trehalose, and imply trehalose protects structures of the cell membrane, mitochondria, and other components.

Clinically, spinal cord ischemia often occurs with clamp/coverage of the T8 to L2 segment, and supraceliac and thoracic aortic clamping in rabbits is ideal for modeling the clinical problem. However, approaching the suprarenal aorta is technically difficult, and rabbits have poor survival after opening the thoracic cavity. The main source of blood flow to the rabbit spinal cord is a segmental supply from the abdominal aorta.[28] Our model of spinal cord ischemia was very reproducible.

We determined the effect of trehalose on blood pressure and blood glucose levels to determine if changes in these parameters might affect spinal cord injury. The time course of blood pressure in the trehalose-administrated group was similar to that in the control group. No hemodynamic effects of trehalose were observed. Blood glucose levels after declamping slightly increased compared with baseline; however, trehalose had no effect on this change.

Several studies have focused on the toxicity associated with trehalose; however, no adverse effects were observed in any of the studies despite the high concentration of trehalose (10%) given intravenously.[29,30] In rabbits and humans, intravenous trehalose is relatively quickly removed from the plasma by renal and other tissue-specific trehalase activity, and there is little accumulation of this sugar.[29,31]

This study has some limitations. First, the concentration of trehalose solution was selected based on a literature review, and the most appropriate concentration and dosing period were not examined. Further investigation to improve the efficacy of trehalose infusion will be necessary.

Second, how trehalose injected into the aorta was delivered to the spinal cord is not clear, and this will also be difficult to determine in the clinical setting. However, multidetector computed tomography has recently shown an arterial communication between the intercostal and lumber arteries and the anterior spinal cord artery, the artery of Adamkiewicz, which supplies blood flow to the spinal cord.[32] In the clinical setting, direct infusion of trehalose solution into the artery of Adamkiewicz may be possible during open surgery via a catheter in an intercostal artery.

Third, although the mechanism of trehalose in spinal cord protection is not clear, our study shows that the protective effect of trehalose against spinal cord ischemia is partly related to the preservation of cell membranes, mitochondria, and other cytoplasmic structures.

Fourth, we investigated only the early phase of spinal cord ischemia. In the future, it will be necessary to examine the protective effect of trehalose in the delayed phase and to compare trehalose with other saccharides.

CONCLUSIONS

Transaortic trehalose infusion was protective against paraplegia, whereas intravenous trehalose reduced spinal cord ischemia in a rabbit model. This study indicated that this method can potentially be a useful adjunct for spinal cord protection during thoracic and thoracoabdominal aortic operations.

AUTHOR CONTRIBUTIONS

Conception and design: ST, MI, KO, TS
Analysis and interpretation: ST, MI, MH, KI, KO, TS
Data collection: ST, MH
Writing the article: ST, MI
Critical revision of the article: KI, KO
Final approval of the article: TS
Statistical analysis: MH, KI
Obtained funding: Not applicable
Overall responsibility: ST

REFERENCES

1. Svensson LG, Crawford ES, Hess KR, Coselli JS, Safi HJ. Experience with 1509 patients undergoing thoracoabdominal aortic operations. J Vasc Surg 1993;17:357-68.
2. Grabitz K, Sandmann W, Stuhmeier K, Mainzer B, Godehardt E, Ohle B, et al. The risk of ischemic spinal cord injury in patients undergoing graft replacement for thoracoabdominal aortic aneurysms. J Vasc Surg 1996;23:230-40.
3. Safi HJ, Campbell MP, Miller CC 3rd, Iliopoulos DC, Khoynezhad A, Letsou GV, et al. Cerebral spinal fluid drainage and distal aortic perfusion decrease the incidence of neurological deficit: the results of 343 descending and thoracoabdominal aortic aneurysm repairs. Eur J Vasc Endovasc Surg 1997;14:118-24.
4. Coselli JS. The use of left heart bypass in the repair of thoracoabdominal aortic aneurysms: current techniques and results. Semin Thorac Cardiovasc Surg 2003;15:326-32.
5. Kotelis D, Geisbusch P, von Tengg-Kobligk H, Allenberg JR, Bockler D. [Paraplegia after endovascular repair of the thoracic and thoracoabdominal aorta]. Zentralbl Chir 2008;133:338-43.
6. Hollier LH, Money SR, Naslund TC, Procter CD, Buhrman WC, Marino RJ, et al. Risk of spinal cord dysfunction in patients undergoing thoracoabdominal aortic replacement. Am J Surg 1992;164:210-4.
7. Bracken MB, Shepard MJ, Collins WF, Holford TR, Young W, Baskin DS, et al. A randomized controlled trial of methylpredonisolone or naloxone in the treatment of acute spinal-cord injury. N Engl J Med 1990;322:1405-11.
8. Kaplan S, Bisleri G, Morgan JA, Cheema FH, Oz MC. Resveratrol, a natural red wine polyphenol, reduces ischemia-reperfusion-induced spinal cord injury. Ann Thorac Surg 2005;80:2242-9.
9. Isaka M, Kumagai H, Sugawara Y, Okada K, Orihashi K, Ohtaki M, et al. Cold spinoplegia and transvertebral cooling pad reduce spinal cord injury during thoracoabdominal aortic surgery. J Vasc Surg 2006;43:1257-62.
10. Hamaishi M, Orihashi K, Isaka M, Kumagai H, Takahashi S, Okada K, et al. Low-dose edaravone injection into the clamped aorta prevents ischemic spinal cord injury. Ann Vasc Surg 2009;23:128-35.
11. Chen Q, Ma E, Behar KL, Xu T, Haddad GG. Role of trehalose phosphate synthase in anoxia tolerance and development in Drosophila melanogaster. J Biol Chem 2002;277:3274-9.
12. Omasa M, Hasegawa S, Bando T, Hanaoka N, Yoshimura T, Nakamura T, et al. Application of ET-Kyoto solution in clinical lung transplantation. Ann Thorac Surg 2004;77:338-9.
13. Hirata T, Fukuse T, Liu CJ, Muro K, Yokomise H, Yagi K, et al. Effects of trehalose in canine lung preservation. Surgery 1994;115:102-7.
14. Tarlov IM. Spinal cord compression: mechanism of paralysis and treatment. Springfield, Ill: Charles C Thomas; 1957. p.147.
15. Wiemken A. Trehalose in yeast, stress protectant rather than reserve carbohydrate. Antonie Van Leeuwenhoek 1990;58:209-17.
16. Crowe JH, Crowe LM, Mouradian R. Stabilization of biological membranes at low water activities. Cryobiology 1983;20:346-56.
17. Emyanitoff RG, Wright BE. Effect of intracellular carbohydrates on heat resistance of Dictyostelium discoideum spores. J Bacteriol 1979;140:1008-12.
18. Liu R, Barkhordarian H, Emadi S, Park CB, Sierks MR. Trehalose differentially inhibits aggregation and neurotoxicity of beta-amyloid 40 and 42. Neurobiol Dis 2005;20:74-81.
19. Chen Q, Behar KL, Xu T, Fan C, Haddad GG. Expression of Drosophila trehalose-phosphate synthase in HEK-293 cells increases hypoxia tolerance. J Biol Chem 2003;278:49113-8.
20. Fukuse T, Hirata T, Nakamura T, Ueda M, Kawashima M, Hitomi S, et al. Role of saccharides on lung preservation. Transplantation 1999;68:110-7.
21. Chen F, Fukuse T, Hasegawa S, Bando T, Hanaoka N, Kawashima M, et al. Effective application of ET-Kyoto solution for clinical lung transplantation. Transplant Proc 2004;36:2812-5.
22. Hopkinson DN, Odom NJ, Bridgewater BJ, Hooper TI. Comparison of saccharides as osmotic impermeants during hypothermic lung graft preservation. Transplantation 1996;61:1667-71.
23. Mouradian R, Womersley C, Crowe LM, Crowe JH. Degradation of functional integrity during long-term storage of a freeze dried biological membrane. Cryobiology 1985;22:119-27.
24. Bando T, Kosaka S, Liu C, Hirai T, Hirata T, Yokomise H, et al. Effects of newly developed solutions containing trehalose on twenty-hour canine lung preservation. J Thorac Cardiovasc Surg 1994;108:92-8.
25. Sakurai M, Nagata T, Abe K, Horinouchi T, Itoyama Y, Tabayashi K. Survival and death-promoting events after transient spinal cord ischemia in rabbits: induction of Akt and caspase3 in motor neurons. J Thorac Cardiovasc Surg 2003;125:370-7.
26. Kiyoshima T, Fukuda S, Matsumoto M, Iida Y, Oka S, Nakakimura K, et al. Lack of evidence for apoptosis as a cause of delayed onset paraplegia after spinal cord ischemia in rabbits. Anesth Analg 2003;96:839-46.
27. Crowe JH, Carpenter JF, Crowe LM. The role of vitrification in anhydrobiosis. Annu Rev Physiol 1998;60:73-103.
28. Zivin JA, DeGirolami U. Spinal cord infarction: a highly reproducible stroke model. Stroke 1980;11:200-2.
29. Riby J, Sunshine S, Kretchmer N. Renal trehalase: function and development. Comp Biochem Physiol 1990;95:95-9.
30. Liu M, Zhang M, Ye H, Lin S, Yang Y, Wang L, et al. Multiple toxicity studies of trehalose in mice by intragastric administration. Food Chem 2013;136:485-90.
31. Richards AB, Krakowka S, Dexter LB, Schmid H, Wolterbeek AP, Waalkens-Berendsen DH, et al. Trehalose: a review of properties, history of use and human tolerance, and results of multiple safety studies. Food Chem Toxicol 2002;40:871-98.
32. Takahashi S, Orihashi K, Imai K, Mizukami T, Takasaki T, Sueda T. Cold blood spinoplegia under motor-evoked potential monitoring during thoracic aortic surgery. J Thorac Cardiovasc Surg 2011;141:755-61.

Submitted May 10, 2013; accepted Jun 29, 2013.